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(to be used for all correspondence after initial filing)

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Filing Date	09 April 2004
First Named Inventor	Robert Harold Bateman
Art Unit	2881
Examiner Name	Bernard E. Souw
Attorney Docket Number	AE-MM-11 (MST-1237 US2)

Total Number of Pages in This Submission   1

ENCLOSURES   *(Check all that apply)*

- [ ] Fee Transmittal Form
  - [ ] Fee Attached
- [ ] Amendment/Reply
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- [ ] Information Disclosure Statement
- [✓] Certified Copy of Priority Document(s)
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  - [ ] Reply to Missing Parts under 37 CFR 1.52 or 1.53

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- [ ] After Allowance Communication to TC
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- [ ] Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- [ ] Proprietary Information
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- Transmittal Letter for submission of a certified copy of a priority document

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Firm Name	Waters Corporation
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Printed name	Jamie H. Rose
Date	February 6, 2007	Reg. No.	45,054

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Typed or printed name	Jamie H. Rose	Date	February 6, 2007

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Bateman et al.	CONFIRMATION NO.:	1303
SERIAL NO.:	10/821,548	GROUP NO.:	2881
FILING DATE:	April 9, 2004	EXAMINER:	Souw, Bernard E
TITLE:	Accurate Mass Measurement		

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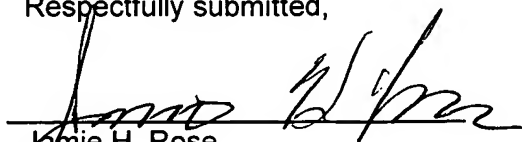
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Sir:

Prior to payment of the issue fee in the above-referenced allowed patent application, Applicants submit herewith, pursuant to 35 U.S.C. 119(b)(3), a certified copy of a priority document, namely U.K. Patent Application No. GB 0308278.1, filed on April 10, 2003.

Applicants believe that no fees are associated with this submission, but the Commissioner is hereby authorized to charge any required fees to Deposit Account No. 23-0503.

Respectfully submitted,

Date: February 6, 2007
Reg. No. 45,054

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Attorney for Applicants

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(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office
Cardiff Road
Newport
Gwent NP9 1RH

1.	Your reference	85.66.80692	
2.	Patent application number *(The Patent Office will fill in this part)*	**0308278.1**	**1 0 APR 2003**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

Micromass UK Limited
Atlas Park
Simonsway
Manchester
M22 5PP

CMK01 6799335-1 000027
P01777700 0.00-0308278.1

Patents ADP number *(if you know it)*

7224827002

If the applicant is a corporate body, give country/state of incorporation

United Kingdom

4. Title of the invention

Mass Spectrometer

5. Name of your agent *(if you have one)*

Frank B. Dehn & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

179 Queen Victoria Street
London
EC4V 4EL

Patents ADP number *(if you know it)*

166001

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*
7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*
a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

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9. Enter the number of sheets for any of the
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    Continuation sheets of this form   0

    Description   10 ✓

    Claim(s)   0

    Abstract   0

    Drawing(s)   4 only ✓

10. If you are also filing any of the following,
    state how many against each item.

    Priority documents   0

    Translations of priority documents   0

    Statement of inventorship and right
    to grant of a patent *(Patents Form 7/77)*   0

    Request for preliminary examination
    and search *(Patents Form 9/77)*   0

    Request for substantive examination
    (Patents Form 10/77)   0

    Any other documents   No
    (please specify)

11.   I/We request the grant of a patent on the basis of this application.

    Signature   Date 10 April 2003

12. Name and daytime telephone number of
    person to contact in the United Kingdom   P.M. Jeffrey
                                               01273 244200

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MICROMASS°

PATENT REQUEST FORM

Department:			IPD case No:	MM1237	
Originator:	Martin R. Green	Signed:		Date:	20 March 2003
Approver:	R.H.Bateman	Signed:		Date:	
Provisional Filing Date:		Proposed Public Disclosure Date:		Date of first offer for Sale: (if known)	

1. Field of application relevant to this invention:

Mass Spectrometry.

2. The State of the art of the current apparatus or methods relevant to this invention:

Mass spectrometers are used to measure and report a value of mass to charge ratio (m/z) of ions. This value is taken to be representative of the sum of the masses of the individual atoms present in an analyte molecule plus or minus the rest mass of an electron, or multiple electrons, depending on the charge state and polarity of the molecule when measured. Mass spectrometers commonly include a computer and software for processing data and for calculating the m/z value of ions. Some such data systems include software for analysing the results of a series of mass measurements, for example, calculating the mean and the standard deviation of a set of mass measurements.

3. The limitations or problems of the current apparatus or methods relevant to this invention:

The accuracy of mass measurement is dependent on the type of mass analyser, the amount of analyte material and the conditions under which a particular mass analyser is operated. An understanding of the errors in mass measurement and the overall confidence in the mass assignment for an ion is useful if this mass assignment is to be used to infer information about the elemental composition of this ion.

Although the state of the art mass spectrometers and associated data system are able to measure mass with high precision and accuracy no software is provided to automatically estimate the accuracy of mass measurement to be expected for individual measurements. Without this information the value or worth of such mass measurements is unknown.

Without an estimate of the accuracy of mass measurement it is not known what range of masses should be considered when trying to calculate elemental compositions that have masses that fall within that range. Alternatively, it is not known what range of masses should be considered when trying to find a match with molecular masses entered in a library, for example a library of peptide masses to be expected from the digestion of a group of proteins.

4. The new invention-Description and explanation of the principles involved:

The invention is the automated computation and reporting of the expected accuracy of individual mass measurements, and describes the methods by which this computation may be carried out. (see attached)

5. The advantages of the new invention over the current apparatus or methods

This data system automatically provides an estimate of the accuracy to be expected for a mass measurement. This provides limits of mass within which proposed elemental compositions must lie. This also provides a measure of confidence in such elemental composition assignments. It provides a means of restricting the range of possible elemental compositions, and guards against over restriction, thereby reducing the possibility of erroneous assignment of elemental composition. It provides a similar advantage when searching against masses in a library of masses.

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6. A description of all known embodiments, including Drawings and a copy of any relevant calculations :
See attached.

7. Any examples (good or bad) of Results or Data from the new Invention:

8. A list of all known literature and patents which describe apparatus or methods similar to that of the invention:

9. People who made a contribution to the invention:

M.R. Green, R.H. Bateman, J. Brown

10. Distribution to:

Phil Jeffrey. Steve Foster.

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4. The new invention-Description and explanation of the principles involved:

1. Introduction

Mass spectrometers are commonly used to measure and report a value of mass to charge ratio (m/z) of an ion. The unit of mass/charge is based on one mass unit being 1/12 of the mass of the most abundant isotope of carbon (^{12}C), and one unit of charge being equal to the charge of an electron. The mass of an ion is the combined mass of the individual atoms present in the ion. This value will also include the rest mass of an electron, or multiple electrons, depending on the charge and polarity of the ion when measured.

The precision of mass measurement is dependent on the scatter of measured values, and the number of measurements. For a normal distribution of measurements the precision may be estimated from the standard deviation (σ) of measurements about the mean and the number of measurements (n). The accuracy of mass measurement is the difference between the mean of the mass measurements and the true mass of the target ion. In general, the more accurate the measurement of m/z ratio the smaller the band of uncertainty, and the more restricted the range of elemental compositions for which their mass falls within this band of uncertainty.

The accuracy of mass measurement is dependent on the type of mass analyser, the amount of analyte material and the conditions under which a particular mass analyser is operated. An understanding of the errors in mass measurement and the overall confidence in the mass assignment for an ion is useful if this mass assignment is to be used to infer information about the elemental composition of this ion

Many mass spectrometers, in particular those employing mass analysers based on fourier transform ion cyclotron resonance, double focusing magnetic sectors, axial and orthogonal time of flight, and RF quadrupole mass filters, are capable of measuring mass to within a few parts per million (ppm) of the true mass. Although such mass spectrometers and associated data system are able to measure mass with high precision and accuracy no software is provided to automatically estimate the accuracy of mass measurement to be expected for individual measurements. Without this information the value or worth of such mass measurements is unknown. However, an understanding of the errors in measurement, and an estimate of confidence in the mass assignment for an individual ion, is essential if this mass assignment is to be used to infer information about the elemental composition of the ion.

This invention is the automated computation and reporting of the expected accuracy of individual mass measurements, and the methods by which the expected mass accuracy for a mass measurement is estimated. This estimation of mass accuracy may be used to automatically restrict the mass window used to calculate proposed elemental composition. Alternatively, the estimation of mass accuracy may be used to set the search window when trying to match the measured mass to those in a library of masses.

2. Methods of estimating mass accuracy

To arrive at a representative value for the precision and the accuracy of an individual mass measurement as many sources of error as possible should be estimated and these errors combined and reported with the measurement.

The sources of error involved with an individual mass measurement are listed below.

a) Statistical errors.

I.   Errors due to ion detection statistics.

II.   Errors due to insufficient sampling frequency.

III.   Computational errors (For example rounding off errors).

IV.   Errors recorded during mass calibration.

In addition to these sources of error there may be specific errors relating to a particular mass measurement which may not be quantified. If these errors are detected no reliable estimation of overall accuracy can be reported.

b) Report exception errors.

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I.   Mass interference

II.   Detector saturation.

III.   Errors due to instability or drift of the instrument since calibration or between calibrations.

a) Statistical Errors

i. Errors due to ion statistics

Often, random error in the determination of m/z value is dominated by ion statistics. A mass spectral peak represents the detection of a number of ions generated in an ion source. An example of such a peak is shown in Figure 1. The Y axis represents intensity, the x axis recorded m/z value. The ion signals detected for a given m/z value are distributed around a mean value and fall within a defined mass envelope. The narrower this envelope of ion arrival the greater is the ability for the mass spectrometer to distinguish between ions differing by small m/z ratios. This is usually expressed as a resolution defined as,

$$R = \frac{M}{\delta M}$$

M = The m/z value corresponding to the mean of the peak envelope.
δM = The width of the envelope in mass units defined in a specific way (for example at the full width half maximum (FWHM)).
R = The mass resolution reported using the same definition.

The mean of this distribution is the estimation of the m/z value for this peak.

If the gain characteristics of the detection / amplification system are known the area of the mass spectral peak may be expressed in terms of absolute number of ions within the peak envelope.

In Figure 2. the m/z scale has been divided into discrete samples. Each sample is described by an index number i. Each index number i is associated with a value of mass M_i. For simplicity it has been assumed that the Y axis represents the absolute number of ions I_i measured at each value of M_i. It is also assumed that each ion detected gives rise to a signal of the same amplitude.

The mean of this distribution M_{mean} is given by

$$M_{mean} = \frac{\sum_i I_i \cdot M_i}{\sum_i I_i}$$

Where

$$\sum_i I_i = N$$

And N is the total number of ions detected.

This mean represents the measured m/z value of the peak. The peak represented in Figure 2 may be considered as a probability distribution with a parent population variance V of ion arrivals which can be estimated from

$$V = \frac{\sum_{all} I_i (M_i - M_{mean})^2}{N - 1}$$

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The standard deviation σ_M around the mean for this parent population is given by

$$\sigma_M = \sqrt{V}$$

From theoretical statistics, if the m/z value is measured based on a single recorded ion belonging to this distribution, the value, M_1 recorded for this ion will be the most likely estimate of the mean of the distribution. The estimated uncertainty in this single measurement is given by σ_M.

If the measurement is repeated for N single ions, an average mean value can be produced. This average mean will have an uncertainty or standard deviation σ_c of

$$\sigma_c = \frac{\sigma_M}{\sqrt{N}}$$

σ_c represents the standard deviation on the measurement of the center of a mass spectral peak with a parent population standard deviation of σ_M and containing N ions.

The expressions above can be related to any peak shape however the shape of a typical mass spectral peak may be approximated by a Gaussian distribution. Assuming a Gaussian distribution and δM defined at full width half maximum (FWHM), referring to Figure 1,

$$\sigma_M = \frac{\delta M}{2.35}$$

This allows the uncertainty in the centroid determination of the peak to be expressed in terms of δM and the number of ions recorded N. The expression below yeilds the uncertainty in the measurement in mass units.

$$\sigma_c = \frac{\delta M}{\sqrt{5.5 \cdot N}}$$

From integration of the Gaussian probability distribution function, approximately 95% of measurements for a given δM and N will fall within +/- $2\sigma_c$. This spread is referred to as 95% confidence limits.

The equations derived above describe the statistical limitations of peak centroid determination in a situation where every ion detected gives an identical response.

Typically mass spectrometers employ multiplier systems to amplify the signal from the individual ions to ensure detection. These multipliers include electron multipliers, photo multipliers, and microchannel plates in a variety of configurations often used in conjunction with electronic amplification devices. Due to the statistical characteristics of some of these devices, ions of the same m/z ratio have a response governed by a separate probability distribution function. When employing an analogue signal recording system (for example an Analogue to Digital Recorder ADC) this distribution leads to an increase in the uncertainty of the determination of the center of the mass spectral peak. This increase may be calculated if the distribution of ion response is well characterized and incorporated into the estimation of error. When using an ion counting system (for example a Time to Digital Recorder TDC) this effect need not be considered.

II Errors due to sampling frequency

In some cases there may be insufficient samples over a mass spectral peak to accurately determine its center. This error should ideally be minimized, however if it is significant it should be incorporated into any final quantification of overall error.

III Computational errors (For example rounding off errors).

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When measuring mass to less than 1/1000 of a mass unit, milli dalton (mda), it is important that calculations are performed and presented with a suitable number of decimal places to avoid unnecessary rounding errors. For example if an ion at m/z 50 is mass measured and the result rounded to four decimal places the possible error due to this rounding will be 50.0000 ± 0.00005. Expressed in parts per million (ppm) this is $50 \pm 1ppm$.

IV Errors recorded during mass calibration.

Calibration involves introducing a reference compound or mixture of reference compounds, which give rise to mass spectral peaks to which precisely known elemental composition and hence m/z value, can be assigned. The measured m/z value for each of these peaks is compared to the known reference m/z values and a suitable calibration function or functions applied. The purpose of this calibration function is to adjust the measured m/z value to be as close to possible to the theoretical mass of the reference compound.

As long as statistical errors are low this procedure will compensate for any systematic errors which are present. Systematic errors in mass measurement are biases in the measured value, which remain the same no matter how many times the measurement is repeated. Any residual errors remaining after calibration may be treated as random errors.

Ideally during calibration enough signal should be acquired so that statistical error due to insufficient ion numbers is negligible. This however may not always be possible.
From the statistical expressions developed above it is possible to assign a value of centroid standard deviation to each peak in the calibration. This may be used to apply appropriate weighting to each m/z value when fitting a calibration function or functions to the data.. This information may be used to predict how errors due to statistics in the initial calibration will affect the uncertainty in the final analyte mass measurement. This is illustrated in figure 5, 6, and 7.

Figure 5 shows the un-calibrated spectum of the reference compound Perfluorotributylamine (PFTBA) acquired in electron impact mode using an orthogonal acceleration time of flight mass spectrometer. The mass range from m/z 300 – 650 is displayed.

In this mass spectrometer m/z value is proportional to the square of the time of flight of the ions. By comparing the square root of the reference m/z value, to the recorded flight time, a calibration expression relating these two values may be generated. This basic relationship was used was used to generate a calibration based on a 3rd order polynomial expression using a weighted least squares method. Figure 6 shows the residual errors after calibration. In figure 6 the X axis represents the calculated m/z value of the spectral peaks based on known their known empirical formula. The Y axis represents the difference between the calibrated m/z values and the known reference m/z values after calibration has been applied. The axis is displayed in parts per million error (ppm). The vertical lines represent $+/- 2\sigma_c$ (σ_c = the statistical uncertainty in the peak center determination in ppm). These error bars represent the calculated scatter, which could be expected on the reported m/z value from repetitive measurements of each calibration point, within a 95% confidence limit.

Figure 7 shows a similar plot but from a much larger data set. The error bars are very small, although still present, and the total error is less than +/- 1ppm over the entire range. The deviation of the calibrated results form the calculated result reflects a remaining element of systematic error. This error may be further reduced by employing a higher order polynomial expression, or alternative curve fitting method.

Because of the variation in signal intensity it can be seen in figure 6 that the error due to statistics within the calibration differs for different mass regions. The errors in this case are dominated by the calculated statistical error on each data point.

In this specific example of calibration method for a time of flight mass spectrometer, coefficients of a third order polynomial of the form

$$\sqrt{M_{ref(i)}} = a + b \cdot \sqrt{M_{est(i)}} + c \cdot (\sqrt{M_{est(i)}})^2 + d \cdot (\sqrt{M_{est(i)}})^3$$

Where
$M_{ref(i)}$ = The calculated m/z value of reference peak I based on the known empirical formula.
$M_{est(i)}$ = The m/z value of reference peak I recorded before calibration.

have been calculated.

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The method involves minimizing the sum of the squared difference between the estimated m/z values $M_{est(i)}$ and the calculated m/z value of the reference material $M_{ref(i)}$ weighted by the calculated statistical variance $\sigma_{c(i)}^2$ of each estimated measurement. Where i is an index denoting individual points in the calibration and N is the total number of points in the calibration. This sum is denoted by the symbol χ^2

$$\chi^2 = \sum_{i=1}^{N} \left[\frac{M_{ref(i)} - f(M_{est(i)})}{\sigma_{c(i)}} \right]^2$$

where

$$f(M_{est(i)}) = a + b \cdot \sqrt{M_{est(i)}} + c \cdot (\sqrt{M_{est(i)}})^2 + d \cdot (\sqrt{M_{est(i)}})^3$$

The coefficients of the polynomial function are adjusted to give the lowest value of χ^2

The χ^2 value gives an overall estimation of the 'goodness of fit' of the calibration expression and can be used to provide a crude acceptance / rejection criteria for a given calibration.

There are many other documented 'goodness of fit tests which may be performed on this type of data depending on the number of calibration points and the calibration approach followed. In principle other methods may be used for rejection or acceptance of calibration.

Using the method of least squares fitting above, the variance in the calculated coefficients in the polynomial expression can be calculated along with the covariance. This information can be used to calculate the variance and hence standard deviation of a measurement of any m/z within the m/z range of this calibration. This standard deviation may be combined with any further statistical information to give an overall estimated error in a subsequent measurement.

Although described for calibration using a least squares n^{th} order polynomial many other calibration schemes may be employed, such as non-linear and linear regression and splines. These may be more suited to particular types of data or mass analysers. In each case it is possible to extract information about the predicted error at particular m/z values based on the statistics of the individual calibrant peaks.

b)   Report exception errors.

There are many sources of systematic error involved with mass measurement, however many of these biases are constant during an experiment and can be removed from the final result by careful characterization of the system. This characterization, or calibration, is essential to ensure that the subsequent measurement is as accurate as possible. If a systematic error is identified and cannot be quantified no reliable estimate of overall accuracy can be presented. The possible source of this error can however be indicated.

I.   Mass interference

Some sources of systematic error may not be constant during the course of a measurement or may not be predicted and corrected during calibration. An example of this is unresolved mass interference.

As an illustration Figure 3 shows a representation of a portion of a mass spectrum from m/z 499.8 to m/z 500.4. For this example the statistical error is assumed to be negligible. Two peaks are shown corresponding to two different chemical species. The two peaks are assumed to have a Gaussian shape. The peak at lowest m/z (P1) has a mean m/z value M1. The peak at highest m/z value (P2) has a mean m/z value of M2. The intensity of P2 is set to be half of the intensity of P1. Both P1 and P1 have a width at half height δM of 0.05 mass units. The mass resolution is therefore 10,000 (FWHM definition). In this example M1 = 500.0000, and M2 500.1500. In this figure it is clear that at this m/z value a resolution of 10,000 is more than sufficient to separate P1 and P2 and enable accurate determination of the mean value of the peak M1 and M2.

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In Figure 4 The seperation between M1 and M2 has been reduced to 0.05 mass units. M1 = 500.0000 and M2 = 500.0500. The two peaks are now clearly not resolved from each other. The resultant single, asymmetrical mass spectral peak shown in, bold line, has a mean m/z value M$_r$ of 500.0167. If P1 represents an analyte ion which is being measured and P2 represents an unexpected unresolved background interference ion, the measurement of M1 has an error E$_{M1}$ where

$$E = M_r - M1$$

In this example E = 0.0167 da

In terms of parts per million (ppm) E = 33.4ppm

Mass interference effects causing systematic error can be recognized, and in some cases approximately quantified, by examination of the mass spectral peak shape compared to an idealized model of the expected peak shape at a particular m/z value. This ideal peak shape may be characterized from that of standard reference peaks prior to a mass measurement. In some cases a Gaussian peak shape can be assumed. If the peak shape or width deviates beyond a pre-set criteria the accuracy of the mass measurement of this m/z value will be poor. The extent to which this effect can be accurately quantified will depend on the sophistication of the peak detection algorithm employed.

II.   Detector saturation.

Another source of systematic error can result when the signal intensity recorded exceeds the operational range of the signal amplification or detection device. In certain mass analysers mass measurement accuracy can be effected by space charge repulsion within the mass analyser itself. These effects can lead to shifts in recorded m/z value due, for instance, to distortions in mass spectral peak shape as the intensity increases. The characteristics of the particular mass analyser and detection electronics utilized must be characterized prior to measurement, and the upper limit of the range at which an appropriate measurement of mass accuracy can be reported found.

III.   Errors due to instability or drift of the instrument since calibration or between calibrations.

An additional source of systematic error, which should be considered, can results from a change in the conditions under which the mass spectrometer is operating relative to those conditions existing when the system was calibrated. If a system parameter has changed, resulting in the calibration becoming invalid, the final mass measurement will be in error. This situation can occur, for instance, because of user intervention, failure of a power supply, interference from an external source of electromagnetic radiation or mass drift caused by ambient temperature changes. The time period which has elapsed between calibration being performed or checked, using a suitable reference compound and a mass measurement being reported should be monitored. The longer this time period becomes the less accuracy can be assigned to the final measurement. Although the error on the final measurement may not be readily quantifiable it is important that effort is made to recognize this situation and reflect a general uncertainty in accuracy with any measurement presented.

Sources of error during mass measurement

To determine an overall figure of accuracy for an individual measurement, possible errors (described above) arising at each step in the mass measurement process should be examined.

Errors recorded in the instrument calibration procedure have been described.

a)   Internal secondary reference correction

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Calibrations are generally made some time before a mass measurement of an analyte is performed. Due to the susceptibility of power supplies to temperature drift and the thermal expansion of materials used in the construction on mass analysers it is necessary to recognise and correct for drift over time.

To ensure that any minor drift in the instrument is accounted for when making a mass measurement an internal reference compound may be introduced at the same time or within a short time period before or after the mass measurement is made. This not only allows the calibration expression to be adjusted for any small change but also provides an internal check of the stability of the system.

For example, in time of flight mass spectrometers a single point 'lock mass' correction is made. A reference material is introduced and the measured m/z value of a single mass spectral peak from this reference material is monitored during the experiment. The empirical formula and hence the calculated m/z value of this internal calibrant is known. Changes in the overall gain of the calibration expression are made on a spectrum to spectrum basis. If more than one internal reference peak is present more complex changes to the overall calibration expression may be incorporated including correction for any offset drift.

An error in the determination of the m/z value of the internal reference will result in a error in the mass measurement of the analyte. All the sources of error previously described should be estimated for the measurement of the internal reference m/z value.

In techniques where the secondary reference material is introduced at exactly the same time as the analyte compound mass interference from background peaks or the analyte peaks may occur. In chromatographic techniques the analyte compound intensity changes with time as a chromatographic peak elutes. The internal reference compound, however, remains at essentially constant intensity. Based on the intensity and the resolution of the internal reference peak the expected short-term scatter in the measured m/z value for this may be calculated. If the m/z value of the internal reference peak changes by significantly more than the calculated short-term statistical scatter it is probable that a mass interference has occurred at the internal reference peak. This could be from mass interference due to the elution of the analyte compound or due to the elution of a contaminant compound unrelated to the analyte. Alternatively, the shift could be due to an instrument parameter change, failure of a power supply, or interference from an external source of electromagnetic radiation. If this shift is greater than the statistical scatter of the internal reference measurement, the resultant error in the accuracy of the analyte peak may be estimated and indicated. Effort must be made to differentiate between long term drift corrected by an internal reference and short term instability causing systematic measurement errors. This mass shift may characteristically occur in a similar time-scale to the chromatographic peak elution width.

b. Analyte peak measurement

All the sources of statistical and possible sytematic error previously described should be estimated for the analyte peak measurement.

In addition, using chromatographic separation techniques, commonly several mass measurements of the analyte peak are made during the chromatographic elution timescale. These are subsequently averaged and presented as a final reported m/z value. It is expected that the individual measurements across the chromatographic peak will have a statistical variation based upon their individual intensities. If a set of mass measurements from a peak from an eluting compound has an exceptionally large spread of values (σ) compared to what is expected it may indicate the presence of interference from co-eluting or partially co-eluting compounds. In this case no reliable estimation of accuracy may be reported.

Combinations of errors

If all the calculated errors above are determined in common units, for instance mass units or parts per million (ppm) in m/z value, the errors may be added in quadrature to yield an overall estimate of the errors associated with a single mass measurement.

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$$\sigma_T = \sqrt{\sigma_1^2 + \sigma_2^2 + \sigma_3^2 \ldots\ldots + \sigma_n^2}$$

Where σ_1 to σ_n represent the standard deviations calculated for each contribution to statistical error. and σ_T is the total calculated standard deviation on the measurement of the analyte ion m/z value.

A description of all known embodiments, including Drawings and a copy of any relevant calculations:

The estimated accuracy of mass measurement is recorded and presented with each m/z measurement displayed or listed. The figure reported represents a scatter in multiples of $\pm \sigma_T$ about the mean value. Based on a gaussian distribution the confidence in the measurement presented may expressed for different multiples of $\pm \sigma_T$. The table below gives some common confidence limits derived from the gaussian distribution.

Range Presented	Probability of measurement falling within the range set
$\pm 0.5\sigma_T$	38.29%
$\pm \sigma_T$	68.26%
$\pm 1.5\sigma_T$	86.64%
$\pm 2\sigma_T$	95.43%
$\pm 2.5\sigma_T$	98.76%
$\pm 3\sigma_T$	99.73%
$\pm 3.5\sigma_T$	99.95%

Generally a figure of $\pm 2.5\sigma_T$ representing 98.76% confidence level is presented.

This figure can appear as annotation within a spectrum or spectrum list and / or incorporated into the output from elemental composition calculation results.

The accuracy of the measurement may also be indicated by automatically setting the number of decimal places for m/z annotation that are presented by the processing software to reflect the calculated confidence limit.

It is important not to indicate too high a level of accuracy especially when restricting the minimum ppm widow used for calculation of proposed elemental composition.. Situations in which insufficient data is available to calculate a meaningful estimation of the overall error in mass measurement should be determined. In the preferred embodiment an estimation of calculated error will only be displayed if

- An internal reference compound was present during the measurement or correction was made within an acceptable time frame.
- The mass calibration used during the measurement was valid. Criteria defining validity of the calibration must be determined for an individual mass spectrometer. These criteria include changes to critical instrument parameters, which could affect calibration, or if an unacceptable time has elapsed since the calibration was performed.
- The mass spectrometer was not operating outside the limits of signal saturation characteristic of the particular analyser or detection system employed.
- The data is within the calibrated m/z range.
- No mass interference was detected.
- No unacceptable instrument instability was detected.

References.

Estimating the Precision of Exact Mass Measurements on an Orthogonal Time of flight Mass Spectrometer. Karl F. Blom. Anal. Chem. 2001,73,715-719

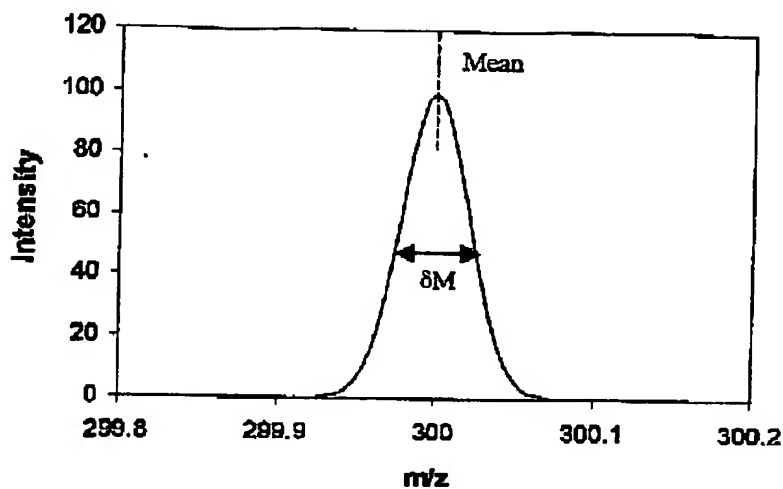Statistics. A Guide to the use of statistical methods in the physical sciences. R.J. Barlow. John Wiley & sons Ltd.

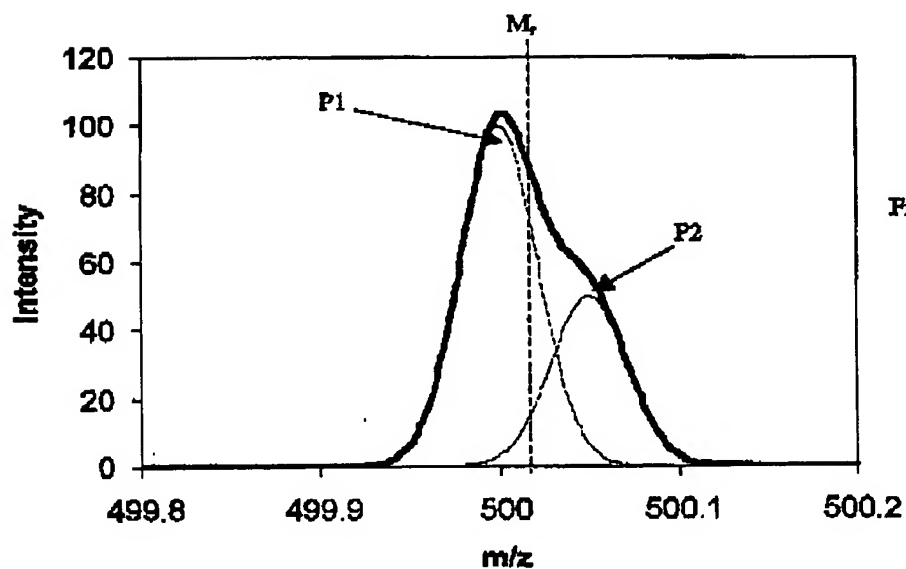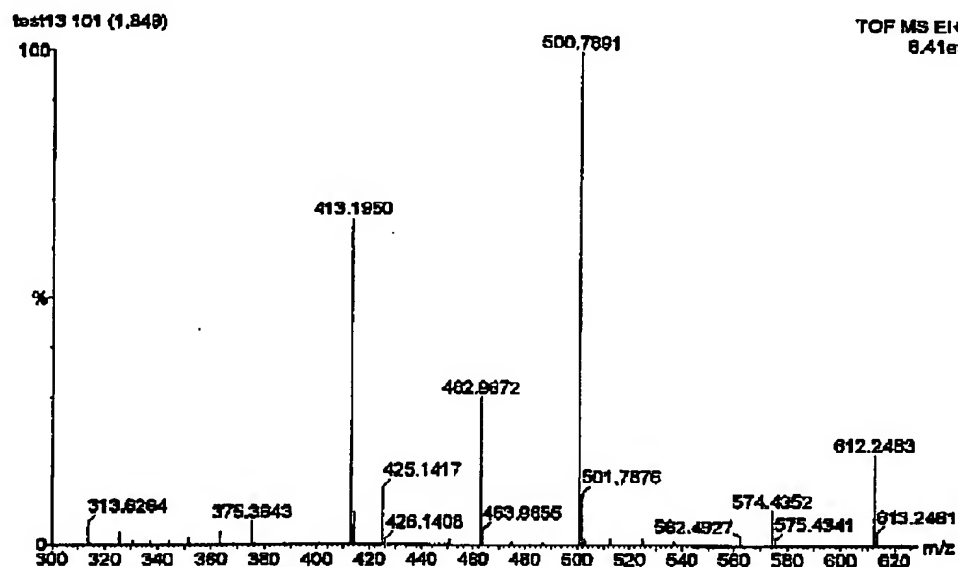0065981 10-Apr-03 03:48

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Figure 1

Figure 2

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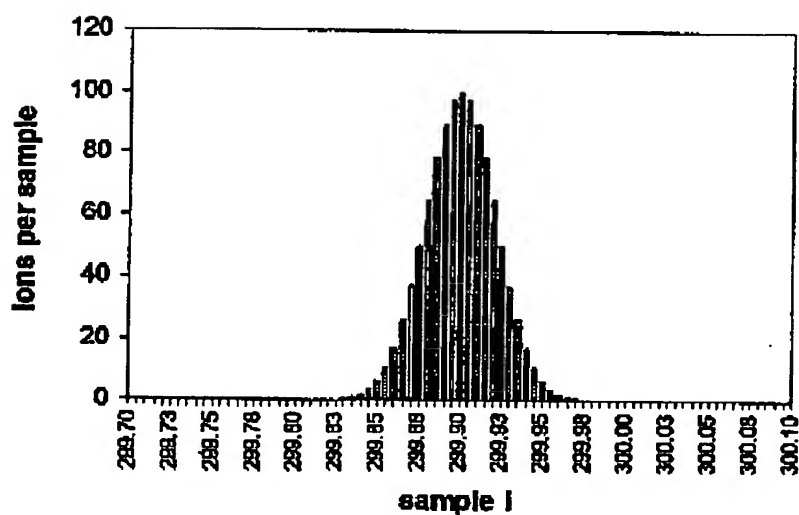
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Figure3

Figure 4

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Figure 5

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Figure 7

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